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Short communication

Indirect chiral separation of tryptophan enantiomers by high performance liquid chromatography with indirect chemiluminiscence detection

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ABSTRACT

In recent years, the study of chiral compounds in vivo has received much attention. In this study, a novel method based on high performance liquid chromatography (HPLC) coupled with chemiluminescence (CL) detection was developed for the separation of tryptophan (Trp) enantiomers. *o*-Phthalaldehyde and *N*-acetyl-L-cysteine were used as chiral derivatization reagents for Trp before it can be detected by HPLC-CL method. The separation was carried out on an ODS column using a mobile phase composed of methanol—0.01 mol/L phosphate buffer (40/60, v/v). Under the optimum conditions, satisfactory results were obtained, including complete separation, good relative standard deviations and low detection limits. The applicability of the proposed method has been validated by determining Trp in biological samples. Linear responses ($r > 0.9990$) were observed over the range of 2.5×10^{-7} to 1.2×10^{-5} g/mL of Trp enantiomers, with quantitation limit of 2.5×10^{-7} g/mL. The assay method shows good specificity to Trp enantiomers, and thus it will have great potential application in clinical diagnosis. The mean extraction efficiency of Trp enantiomers in mice plasma samples were 98.48% and 97.40%, respectively. The mean relative standard deviation (RSD) of Trp enantiomers were <3%.

1. Introduction

In recent years, chemiluminescence (CL) as a detection technique of high performance liquid chromatography (HPLC) is very attractive due to higher sensitivity, good selectivity, wide linear ranges and simpler instrumentation [1,2]. The HPLC-CL method has been successfully applied to the determination of various compounds, including doxorubicin [3], kaempferol [4], rutin and quercetin [5]. No previous studies have hitherto attempted to extend the application scope of the HPLC-CL method to the separation of chiral compounds, such as amino acids, hypoglycemic agents and anti-inflammatory drugs.

Tryptophan is an essential constituent of proteins and precursor of melatonin and serotonin, which improve the sleep, mood and mental health. Due to their optical isomers present different activity, toxicity and metabolic route, it is very necessary to separate Trp enantiomers. Some techniques have been explored for discrimination of Trp enantiomers, such as HPLC [6,7], CE [8] and the electrochemical method [9]. Although the reported techniques are useful, these are either low sensitivity or expensive equipments. Therefore, a simple and reliable method for the chiral separation of Trp is highly desirable. Based on the advantages of HPLC-CL and the CL inhibition by Trp enantiomer derivatives of the Luminol–KSCN system in alkaline medium, the HPLC-CL method was applied to the separation of Trp enantiomer derivatives Fig. 1 was the derivatization scheme of tryptophan. Importantly, the method of HPLC with CL detection for the separation of Trp has not been reported until now.

In this study, a postcolumn Luminol–KSCN detector for HPLC was established for the separation of Trp enantiomer derivatives. The conditions for HPLC separation and CL detection were optimized. As a preliminary application, the proposed method was applied to the determination of D-/L-Trp in vivo. To the best of our knowledge, there has been no report on HPLC-CL method used for the determination of D-/L-Trp in biological samples.

2. Experimental

2.1. Reagents

D-/L-Tryptophan were purchased from Zhengzhou Dingguo Biotechnology Co., Ltd., (Zhengzhou, China). $Na_4B_2O_7{\cdot}10H_2O$,

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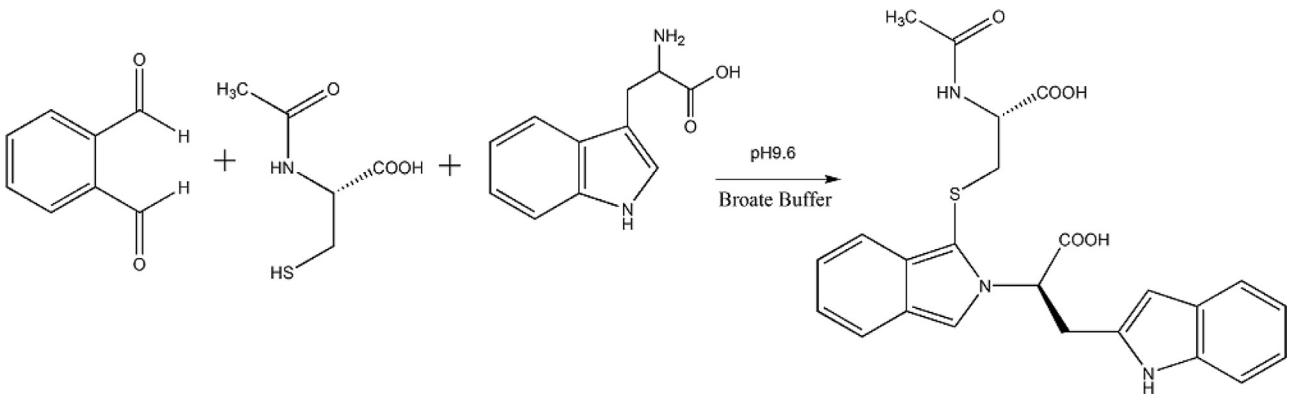

Fig. 1. The derivatization scheme of tryptophan.

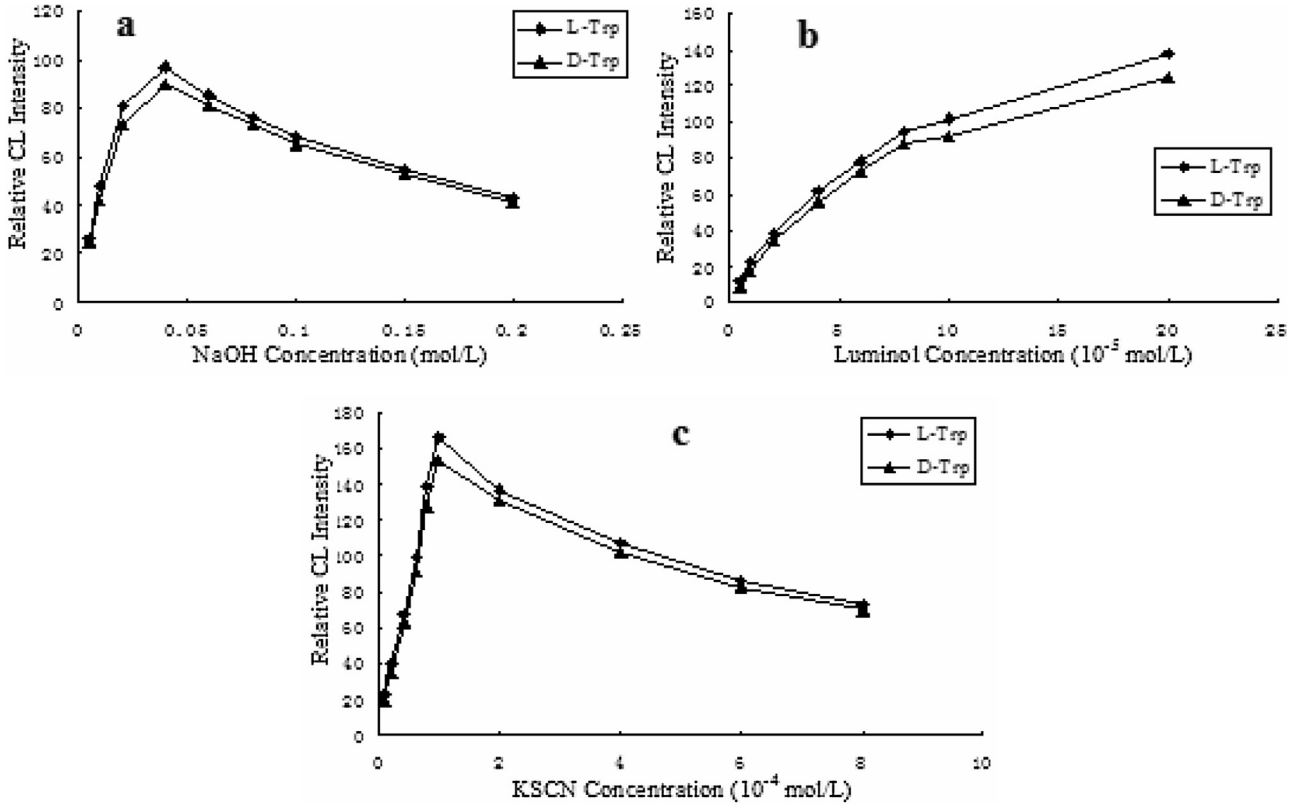

Fig. 2. Effect of NaOH, Luminol and KSCN concentration on the relative CL intensity.
Reaction conditions: (a) Luminol, 5.0×10^{-5} mol/L; KSCN, 5.0×10^{-5} mol/L. (b) NaOH, 0.04 mol/L; KSCN, 5.0×10^{-5} mol/L. (c) NaOH, 0.04 mol/L; Luminol, 8.0×10^{-5} mol/L.

KH_2PO_4 and NaOH were obtained from Tianjin Kermel Chemical Reagent Co., Ltd., (Tianjin, China). o-Phthalaldehyde (OPA) and *N*-acetyl-L-cysteine (NAC) were ordered from Aladdin Industrial Co., (Shanghai, China).

A stock solution of Luminol (1.0×10^{-2} mol/L) was prepared by dissolving Luminol (Beijing Solarbio Technological Co., Ltd., Beijing, China) in 0.10 mol/L NaOH solution and stored at least one week before use. KSCN stock solution (1.0×10^{-2} mol/L) was prepared by dissolving 97.18 mg of KSCN (Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China) in 100 mL ultrapure water and stored in the dark. Methanol was of HPLC grade, and all other reagents were of analytical reagent grade. The ultrapure water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). The mobile phases of HPLC were freshly prepared and filtered through a 0.45 μm membrane filter (Bandao, Shanghai, China), and then degassed prior to use.

2.2. Apparatus

The HPLC-CL detection system consisted of HPLC separation system and post-column CL detection system. The HPLC system was Agilent 1100 series (Agilent Technologies Co., Ltd., USA), including a quaternary pump, a vacuum degasser, a column oven, a manual sample valve injector with a 20 μL loop, and an analytical column (Shim-pack ODS, 150 mm × 4.6 mm i.d. 5 μm; Shimadzu, Japan). The CL detection was conducted on a flow injection CL system (Xi'an Remax Analytical Instrument Co., Ltd., Xi'an, China), consisting of a model IFFM-E peristaltic pump, a six-way injection valve (fitted with a 100 μL sample loop), a mixing tee, a model IFFS-A CL detector equipped with a glass coil (used as reaction coil and detection cell) and a photomultiplier tube (PMT). The CL signal was recorded by using an IBM-compatible computer, equipped with a data acquisition interface.